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Food preference in Drosophila robusta and Drosophila tripunctata following desiccation

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Introduction

Drosophila tripunctata and *Drosophila robusta* are local species trapped on the University of Northern Iowa preserve off of University Avenue. *Drosophila* literally means "lover of dew". *Tripunctata* means "three points", it has 3 points on its abdomen. *Robusta* means "robust", of which this species definitely is. *D. tripunctata* are a small species, which leaves them with a low volume/surface area ratio. This means they are more susceptible to environmental extremes, particularly the loss of water. *D. robusta* are a large and "robust" fly, and they are more tolerant of such environmental hardships. "Desiccation" is drying out, or to remove moisture. This is done with flies by removing food, which is also their source of water. Food also is the site of egg laying for females, and may provide an alternative attraction to a specific bait.

Food preference and choice of oviposition (egg laying) sites are critical factors for insects such as *Drosophila* and effect many aspects of their lives (Jennings and Seager, 1982). Different species of *Drosophila* fill various niches by making use of a variety of foods, which also affects their seasonal abundances (Shorrocks, 1983). To survive together, *Drosophila* species must somehow divide up their habitat and resources. Species with similar ecological requirements can coexist only if they are able to partition a habitat effectively and thus reduce competition (Hardin, 1960). *Drosophila* survival and reproduction may thus depend on the ability to choose appropriate microhabitats (McDaniel *et al.*, 1995), including the ability to locate and discriminate among different food resources (Turelli *et al.*, 1984).

Bobinet (unpublished data) found that *D. robusta* preferred banana bait in the wild at a statistically significant level ($p<0.05$), while *D. tripunctata* preferred mushroom bait in the wild at a statistically significant level ($p<0.05$). These data were supported by further trappings done in Cedar Falls, Iowa (Seager *et al.*, unpublished). Doolittle (unpublished data) successfully replicated these bait preferences in the laboratory.

Methods

The apparatus was 1 cm high, 10 cm wide, and 80 cm long. It was made out of Plexiglas and coated with Teflon. The apparatus was divided into sections that were partitioned off with Plexiglas gates. The 20 cm on each end was blocked off, and not used in the experiments, leaving 40 cm of experimental apparatus. The 40 cm was sectioned into 7 compartments, 6 equally sized, with the middle compartment being twice the size of a smaller one. Holes 2.5 cm in diameter were on the lid and covered with nylon netting. The bait scent was blown in through the holes on the extreme ends of the apparatus. The banana bait was blown in on the extreme left, while the mushroom bait was blown in on the extreme right of the apparatus. Tubing was used to blow air in from the bait source through the holes covered with netting.

The flies were classified by the location where they were found after the 2 hour trial was over. Those flies found in the 2 compartments nearest the banana bait were termed "banana responders". Those flies found in the 2 compartments nearest the mushroom bait were termed "mushroom responders". Those flies that were found in the middle 3 compartments were termed "non-responders".

The baits and the apparatus remained consistent with the field study and with Doolittle's research. The baits were prepared 3 days prior to the trial in

which it was used. The banana bait consisted of 100 grams of banana homogenized with 1.25 grams of bakers yeast. The mushroom bait consisted of 65 grams of mushrooms homogenized with 15 grams of water.

The flies used were from isofemale lines established in the Seager and Doolittle research. Each isofemale line (9 lines for each species) was started from a single female that had been inseminated in the wild and captured in Cedar Falls, Iowa. *D. robusta* and *D. tripunctata* were used because they both illustrated a clear bait preference and they are easily maintained. *D. robusta* were found solely (100%) on the banana bait in the wild. *D. tripunctata* were found primarily (~80%) on the mushroom bait. Doolittle's research successfully replicated this in the laboratory, confirmed by the desiccation trials. Doolittle found that although the species were indeed attracted to one bait over the other, a major population of flies made no preference, the non-responders. This high incidence of non-responders was the focus of the desiccation work. Two hypothesis were made:

1. The non-responders were really insignificant, they would not have been found in nature at all.

2. The flies were not hungry/thirsty enough. Desiccating the flies may make them hungry/thirsty and force them to make a food choice.

This research attempted to force the flies into making a food choice by desiccation. The flies were placed into viles without food or water, and with a desiccant present. The flies were kept at a humidity of 15% + 5% for 7 hours in a growth chamber. 7 hours was determined experimentally because *D. tripunctata* showed a significant death rate after 7 hours.

Order of events in desiccation:

3-5 days prior to trials: Clear flies out of bottles. Flies that emerge in the next 2-3 days will be sorted by sex. This allows for a consistent age group to be tested. The flies are then placed in vials containing food. This allows the flies time to recover from being anesthetized with carbon dioxide which allows them to be easily sorted by sex. 80-100 flies were placed into the apparatus for each trial, with some of those dying or escaping. Thus 10-12 flies should be taken from each of the isofemale lines to maintain a representative sample.

3 days before trials: Prepare baits
---Banana bait=100g banana + 1.25 g yeast---homogenize.
---Mushroom bait=65g mushroom + 15 ml water---homogenize.

7 hours before trial: Remove flies from food vials to smaller vials without food. These vials are sealed off with nylon netting and a rubber band and placed in a desiccation box with desiccant (Dry-Rite). The desiccant will remove moisture from the air. The desiccant is blue when it can absorb moisture, and turns purple when it cannot. The moisture can be removed from the desiccant by placing in an oven.

Trial time: Turn on the air to blow the bait scent into the apparatus. Remove the flies from their viles using a mouth aspirator made of tubing, a cut pipette, and cheesecloth. Place the flies into the trial apparatus through the center hole by lifting the nylon covering. Raise the gates dividing the compartments.

2 Hours Later: Return. Close the gates. Turn on the carbon dioxide. Raise the lid and remove the flies, counting the number of flies in each compartment. Wipe out apparatus with a paper towel to remove any liquid that may have been blown through.

Note: This procedure is altered from Doolittle's procedure only by the 7 hours of desiccation. Separate trials are used for each sex of a species, thus 4 groups each requiring 5 trials of desiccation totaled 20 desiccation trials.

Results and Discussion

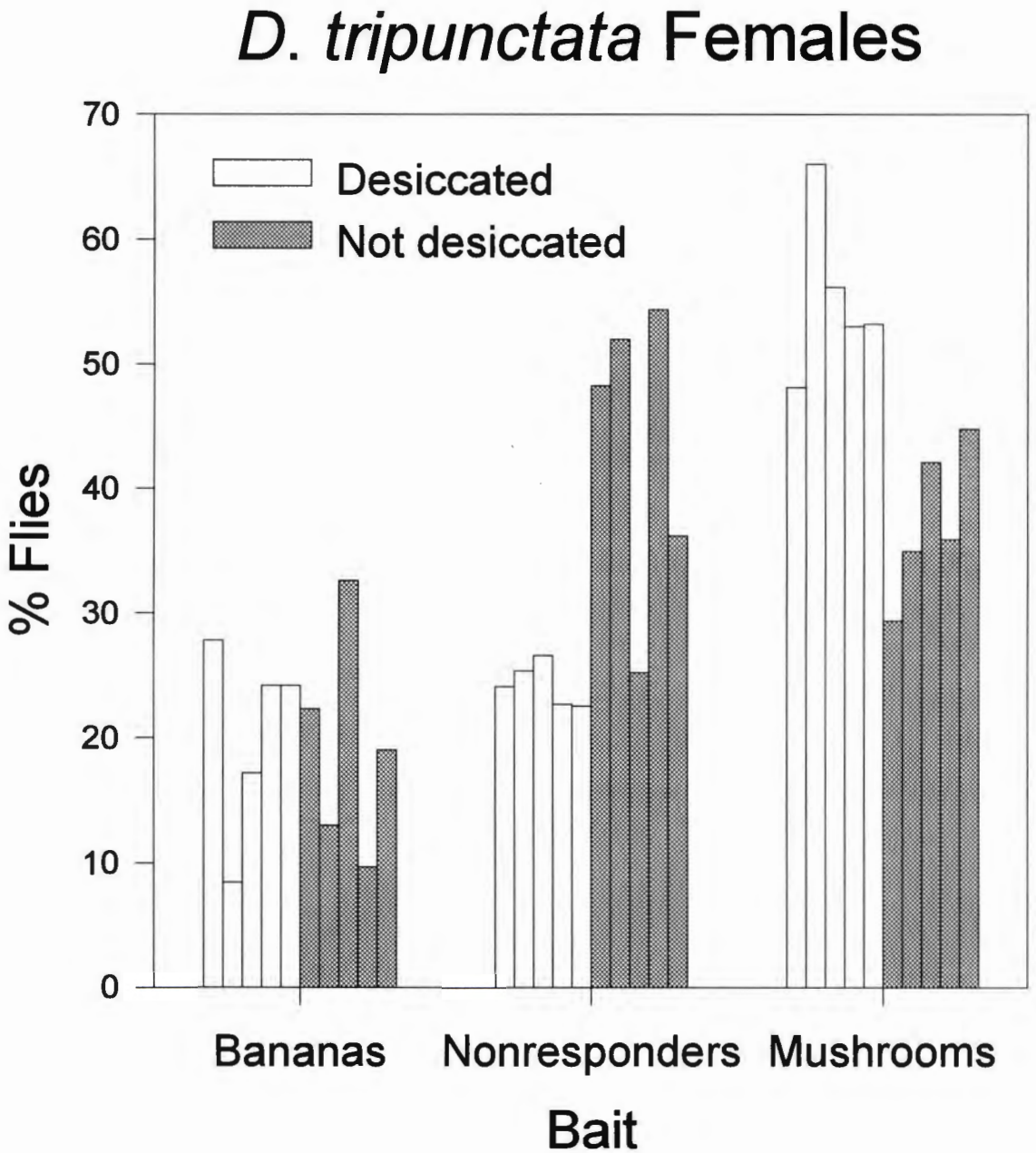
The desiccation data was analyzed vs. the non-desiccated data (Doolittle's) using a Generalized Linear Models (GLIM) software. GLIM confirmed that *D. robusta* and *D. tripunctata* are indeed making different food choices, both in the non-desiccated and desiccated trials. Males and females of the same species do not seem to be statistically different.

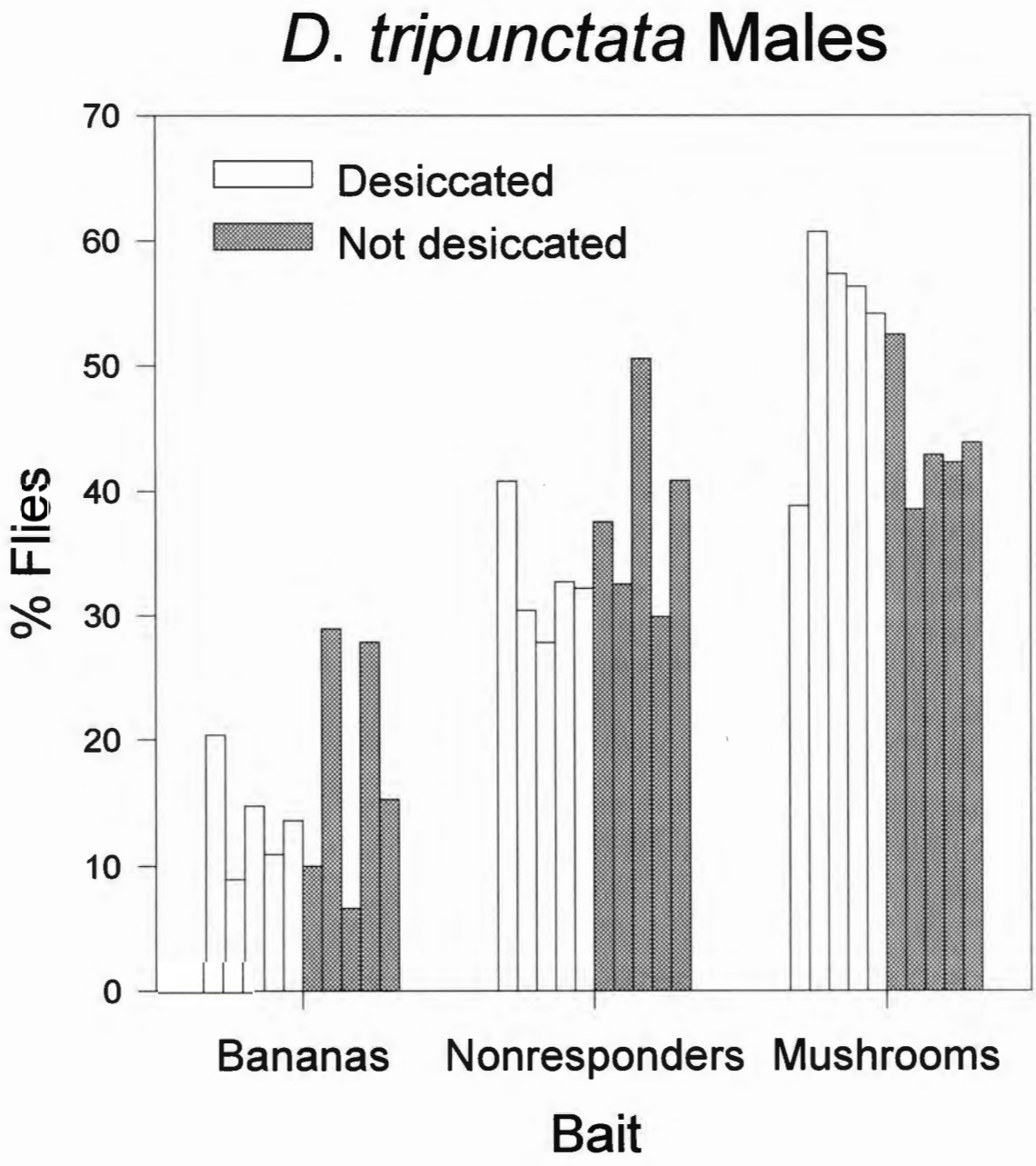
GLIM also confirmed that the desiccation of *D. tripunctata* significantly lowered the rate of non-responders, and that their was an increased preference for

mushrooms when desiccated. GLIM could not confirm that desiccation significantly changed the food preference of *D. robusta* or that the rate of non-responders had been changed.

The resistance of *D. robusta* to desiccation may offer a future research opportunity. Desiccating *D. robusta* for a longer period of time may bring the significant changes observed with *D. tripunctata*. Other options may be to grow the flies on an altered food source. Growing *D. tripunctata* on a banana mixture was performed. 2 parts Carolina food, 1 part water, and 1 part banana *allowed D. tripunctata* to reproduce very well. Raising *D. tripunctata* on banana may alter their response to the banana bait, which would suggest an environmentally influenced trait or conditioned response.

A selection experiment was also briefly attempted. Running trials, and selecting only certain responders to breed the next generation can be tested. If after generations of selection, the bait preference is shifted, then this would suggest a genetic component to the bait preference.


D. tripunctata Females
70
60
50
40
30
20
10
0
% Flies
Desiccated
Not desiccated
Bananas
Nonresponders
Mushrooms
Bait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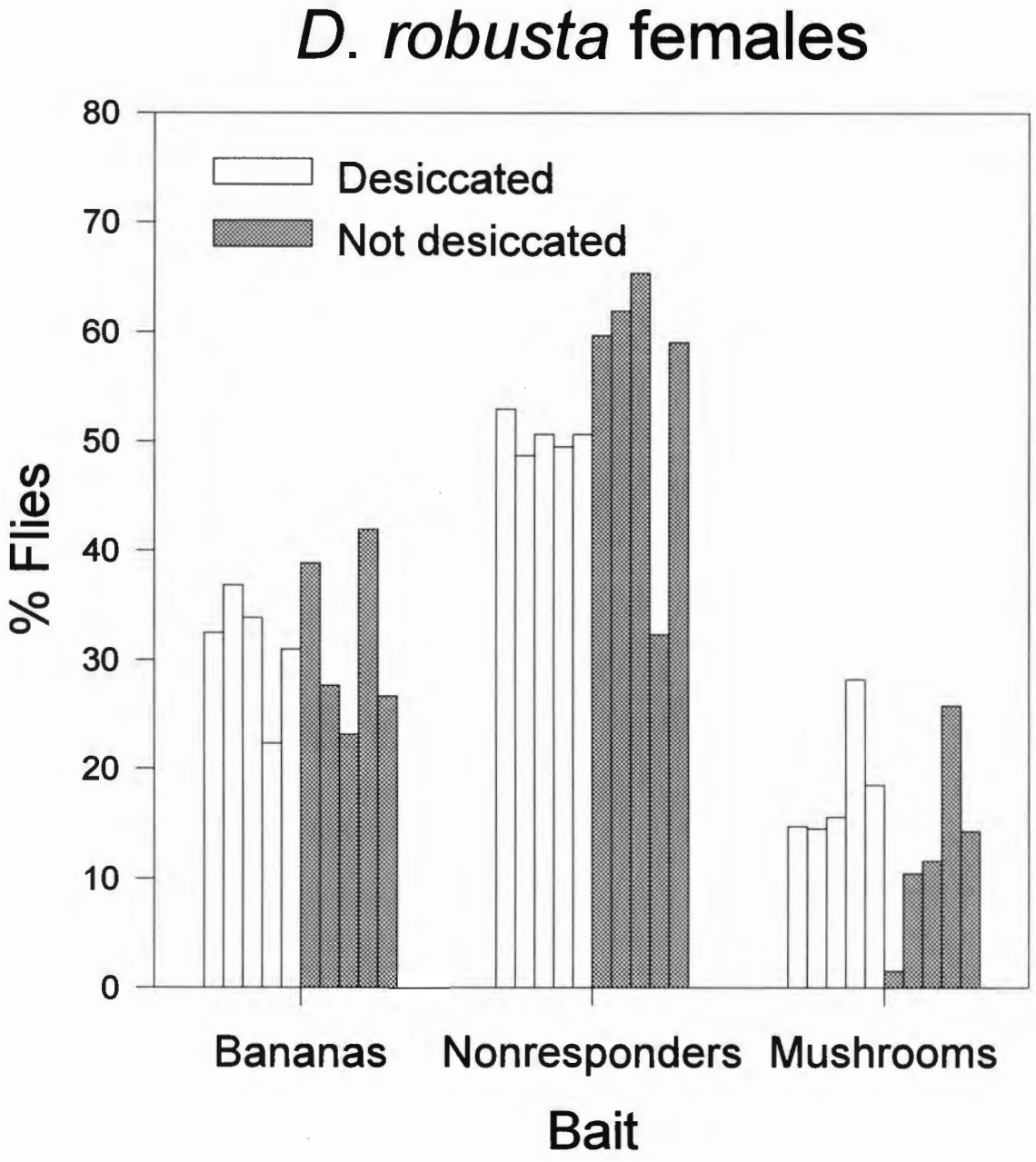
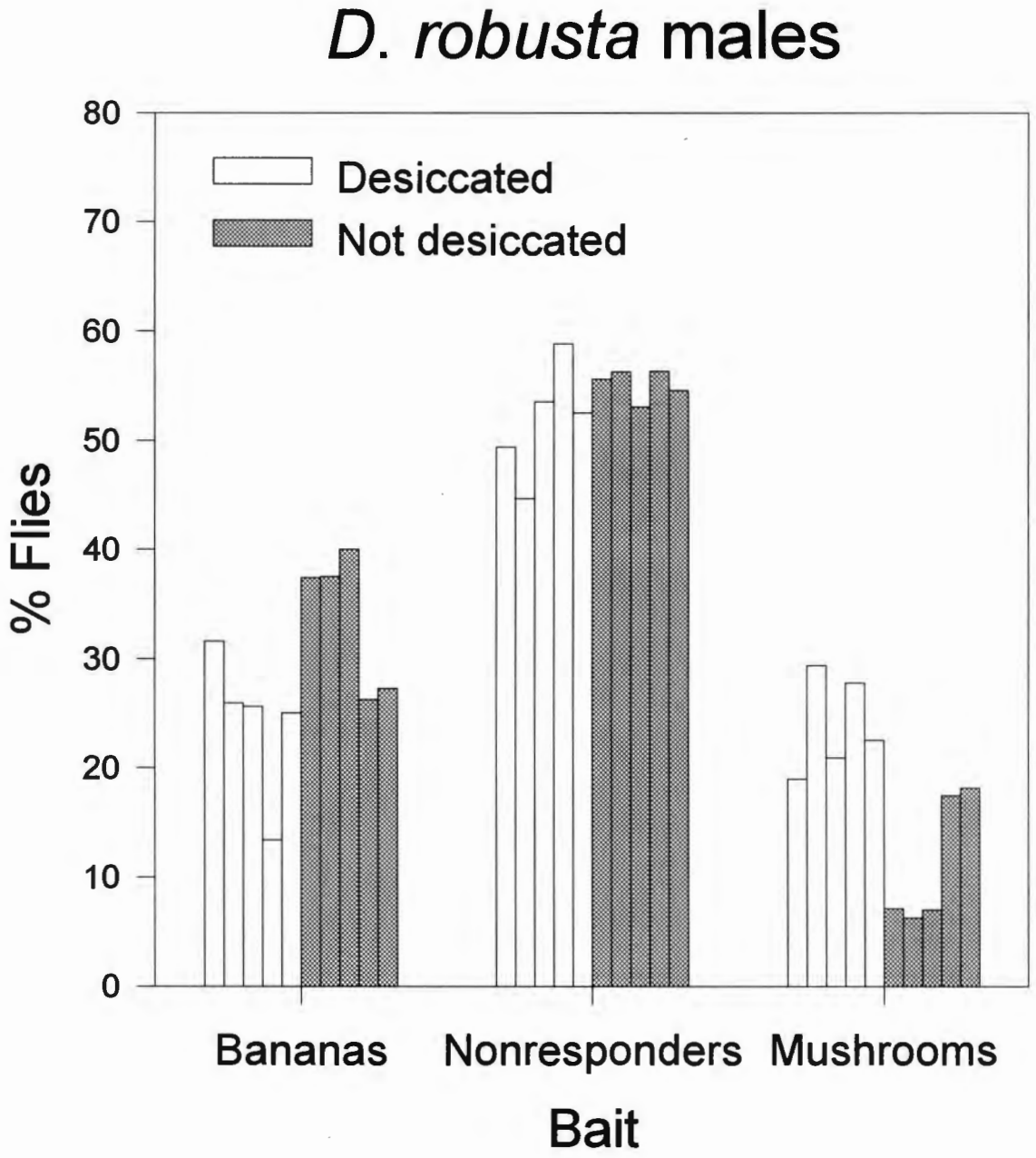

D. robusta females
Desiccated
Not desiccated
% Flies
80
70
60
50
40
30
20
10
0
Bananas
Nonresponders
Mushrooms
Bait


D. robusta males
80
70
60
50
40
30
20
10
0
% Flies
Desiccated
Not desiccated
Bananas
Nonresponders
Mushrooms
Bait

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